

Supplementary Table 1. Data collection and refinement statistics for TRIM24 PHD-Bromo and its complexes with histone peptides.

Data collection and refinement statistics					
Crystal	Trim24 PHD-Bromo (824-1006)	Trim24 PHD-Bromo with H3(1-10)	Trim24 PHD-Bromo with H3K23ac(13-32)	Trim24 PHD-Bromo with H3K27ac(23-31)	Trim24 PHD-Bromo with H4K16ac(14-19)
Beam line	APS-24ID-E	APS-24ID-E	APS-24ID-E	Brookhaven X29	APS-24ID-E
Wavelength	0.97918	0.97918	0.97918	1.08090	0.97918
Space group	<i>P</i> 1	<i>P</i> 1	<i>P</i> 2 ₁ 2 ₁	<i>C</i> 2	<i>C</i> 2
Unit cell					
a, b, c (Å)	36.5,48.5,122.7	35.7,63.8,79.3	36.8,41.1,126.0	89.6,36.5,128.8	89.8,36.3,127.0
A, β, γ (°)	86.5, 81.5, 67.8	89.9, 90.0, 89.8	90.0, 90.0, 90.0	90.0,110.0,90.0	90.0, 109.7, 90.0
Resolution (Å)	50-2.00 (2.07-2.00) ^a	50-2.00 (2.07-2.00)	50-1.90 (1.97-1.90)	50-1.76 (1.82-1.76)	50-1.70 (1.73-1.70)
R _{sym}	0.107 (0.510)	0.092 (0.491)	0.064(0.264)	0.059 (0.460)	0.089 (0.445)
I/σ (I)	26.2 (4.0)	28.1 (3.9)	46.1 (4.6)	28.0 (2.3)	22.1 (2.2)
Completeness (%)	96.9 (96.8)	98.8 (99.5)	98.8 (91.9)	99.0 (91.0)	98.6 (94.5)
Redundancy	3.9 (4.0)	3.7 (3.8)	8.0 (5.7)	5.0 (4.6)	5.7 (5.3)
Number of unique reflections	50497	47703	15683	38897	42284
R _{work} /R _{free} (%)	22.1 /24.5	22.7/25.4	21.7 /24.1	20.6 /22.5	19.1 /21.6
Number of non-H atoms					
Protein	5739	6064	1529	2945	2964
Water	242	236	91	248	342
Other ligands	8	8	17	4	4
Average B factors (Å ²)					
Protein	35.9	42.6	35.4	29.5	24.5
Water	36.7	36.6	33.9	32.9	31.4
Other ligands	31.2	47.3	38.8	27.9	16.6
R.m.s. deviations					
Bond lengths (Å)	0.006	0.006	0.007	0.006	0.005
Bond angles (°)	1.27	1.31	1.36	1.24	1.32

^a Highest resolution shell (in Å) shown in parentheses.

Supplementary Table 2. ITC-based binding parameters for complex formation between different histone peptides and wild-type or mutants of TRIM24 PHD-Bromo/Bromo constructs.

Peptide ¹	Protein Sample	K_D (μ M)	ΔH (kcal/mol)
H3(1-15)K4	PHD-Bromo (WT)	8.6 ± 0.4	-4.48 ± 0.03
H3(1-15)K4me1	PHD-Bromo (WT)	41 ± 2	-2.74 ± 0.03
H3(1-15)K4me2	PHD-Bromo (WT)	198 ± 26	-2.8 ± 0.3
H3(1-15)K4me3	PHD-Bromo (WT)	> 400	Not determined
H3(1-15)K4	PHD-Bromo (D827A)	133 ± 5	-4.4 ± 0.1
H3(1-15)K4	PHD-Bromo (C840W)	> 400	Not determined
H3(13-32)K23ac-Bio*	PHD-Bromo (WT)	8.8 ± 0.1	-11.49 ± 0.02
H3(13-32)K27ac-Bio	PHD-Bromo (WT)	206 ± 44	-9.8 ± 1.8
H3(13-32)K23ac-Bio	PHD-Bromo (F979A/N980A)	> 400	Not determined
H3(13-32)K23ac-Bio	PHD-Bromo (E981A)	26 ± 1	-9.6 ± 0.2
H3(1-20)K9ac	Bromo	232 ± 33	-4.6 ± 0.7
H3(1-19)K14ac	Bromo	229 ± 32	-8.2 ± 1.4
H4(1-20)K16ac	PHD-Bromo	26 ± 2	-8.7 ± 0.2
H3(1-33)K4K23ac-Bio	PHD-Bromo (WT)	0.096 ± 0.007	-12.53 ± 0.04
H3(1-33)K4me3K23ac-Bio	PHD-Bromo (WT)	0.56 ± 0.04	-9.47 ± 0.05
H3(1-33)K4-Bio	PHD-Bromo (WT)	2.3 ± 0.2	-1.93 ± 0.02
H3(1-33)K4K23ac-Bio	PHD-Bromo (C840W)	0.60 ± 0.03	-10.19 ± 0.05
H3(1-33)K4K23ac-Bio	PHD-Bromo (F979A/N980A)	0.68 ± 0.07	-4.63 ± 0.04
H3(1-33)K4K23ac	PHD-Bromo (WT)	0.070 ± 0.010	-10.23 ± 0.06
H3(1-33)K4me3K23ac	PHD-Bromo (WT)	0.34 ± 0.04	-8.58 ± 0.07
H3(1-33)K4	PHD-Bromo (WT)	1.4 ± 0.3	-1.71 ± 0.03
H3(1-33)K4K23ac	PHD-Bromo (C840W)	0.52 ± 0.05	-9.2 ± 0.06
H3(1-33)K4K23ac	PHD-Bromo (F979A/N980A)	0.71 ± 0.07	-4.44 ± 0.03

* Bio indicates biotin-labeling at the C-terminus.

¹ITC binding studies with long H3(1-33) peptides with/without modifications on K4 and K23 were first undertaken on C-terminally biotin-labeled peptides, but were independently confirmed using peptides that lacked this C-terminal modification.

Supplementary Table 3. Fluorescence Polarization (FP) based dissociation constants between different histone peptides and wild-type or mutants of TRIM24 PHD-Bromo.

Protein Sample	Peptide	K_D (μ M)
PHD-Bromo (WT)	H3(1-33) Um	4.2 ± 2.0
PHD-Bromo (WT)	H3(1-33) K23ac	0.185 ± 0.027
PHD-Bromo (WT)	H3(1-33) K4me3K23ac	2.4 ± 1.1
PHD-Bromo (C840W)	H3(1-33) Um	Not detected
PHD-Bromo (C840W)	H3(1-33) K23ac	3.2 ± 0.9
PHD-Bromo (C840W)	H3(1-33) K4me3K23ac	8.7 ± 5.3
PHD-Bromo (F979A/N980A)	H3(1-33) Um	1.8 ± 0.3
PHD-Bromo (F979A/N980A)	H3(1-33) K23ac	5.7 ± 1.8
PHD-Bromo (F979A/N980A)	H3(1-33) K4me3K23ac	Not detected

Supplementary Table 4. Biological functions and representation of TRIM24 gene targets

-E₂ Genes - Biological Functions		
Category	Gene Number	Percent
transcription	1631	32.4
metabolic process	1196	23.8
biosynthetic process	597	11.9
cell death	560	11.1
development	317	6.3
chromosome organization	164	3.3
cellular component organization	134	2.7
protein complex assembly	132	2.6
differentiation	130	2.6
vesicle-mediated transport	76	1.5
hormone secretion	73	1.5
gene silencing	18	0.4
+E₂ Genes - Biological Functions		
Category	Gene Number	Percent
transcription	2724	34.8
metabolic process	1601	20.5
biosynthetic process	944	12.1
cell death	430	5.5
protein complex assembly	364	4.7
cell cycle	342	4.4
kinase activity	341	4.4
signal transduction	323	4.1
cell development	270	3.5
chromosome organization	208	2.7
hormone response	198	2.5
growth	79	1
Common Genes - Biological Functions		
Category	Gene Number	Percent
transcription	562	34.9
metabolic process	318	19.8
biosynthetic process	199	12.4
development	152	9.4
cell death	145	9
chromosome organization	98	6.1
differentiation	54	3.4
growth	29	1.8
intracellular transport	27	1.7
response to hormone stimulus	25	1.6

Supplementary Table 5. TRIM24 targets genes associated with breast cancer in estrogen-depleted MCF7 cells

HER2 Signaling in Breast Cancer Gene List	
Gene Symbol	Gene Name
CCNE1	G1/S-specific cyclin-E1
ERBB3	Receptor tyrosine-protein kinase erbB-3
ITGB4	Integrin beta-4
GSK3A	Glycogen synthase kinase-3 alpha
PRKCE	Protein kinase C epsilon type
CDKN1A	Cyclin-dependent kinase inhibitor 1
MDM2	E3 ubiquitin-protein ligase Mdm2
RAC1	Ras-related C3 botulinum toxin substrate 1
KRAS	GTPase KRas
MRAS	Ras-related protein M-Ras
PRKCD	Protein kinase C delta type

Hereditary Breast Cancer Signaling Gene List	
Gene Symbol	Gene Name
CREBBP	CREB-binding protein
CDK1	Cell division control protein 2 homolog
DDB2	DNA damage-binding protein 2
HDAC6	Histone deacetylase 6
CDKN1A	Cyclin-dependent kinase inhibitor 1
RAC1	Ras-related C3 botulinum toxin substrate 1
PALB2	Partner and localizer of BRCA2
MSH6	DNA mismatch repair protein Msh6
POLR2G	DNA-directed RNA polymerase II subunit RPB7

KRAS	GTPase KRas
MRAS	Ras-related protein M-Ras
POLR2H	DNA-directed RNA polymerases I, II, and III subunit RPABC3
Estrogen-Dependent Breast Cancer Signaling Gene List	
Gene Symbol	Gene Name
CREB3L4	Processed cAMP-responsive element-binding protein 3-like protein 4
FOS	Proto-oncogene protein c-fos
RAC1	Ras-related C3 botulinum toxin substrate 1
KRAS	GTPase KRas
MRAS	Ras-related protein M-Ras
IGF1R	Insulin-like growth factor 1 receptor beta chain

TABLE 6: TRIM24 TARGET GENES ASSOCIATED WITH BREAST CANCER – ESTROGEN-TREATED MCF7 CELLS

HER2 Signaling in Breast Cancer Genes	
Gene Symbol	Gene Name
BAD	Bcl2 antagonist of cell death;BAD
CDC42	Cell division control protein 42 homolog;CDC42
CCND1	G1/S-specific cyclin-D1;CCND1
CCNE1	G1/S-specific cyclin-E1;CCNE1
CCNE2	G1/S-specific cyclin-E2;CCNE2
ERBB3	Receptor tyrosine-protein kinase erbB-3;ERBB3
ITGB8	Integrin beta-8;ITGB8
RAC3	Nuclear receptor coactivator 3;NCOA3
PRKCE	Protein kinase C epsilon type;PRKCE
PRKCZ	Protein kinase C zeta type;PRKCZ
CDKN1A	Cyclin-dependent kinase inhibitor 1;CDKN1A
CDKN1B	Cyclin-dependent kinase inhibitor 1B;CDKN1B
MDM2	E3 ubiquitin-protein ligase Mdm2;MDM2
RAC3	Ras-related C3 botulinum toxin substrate 3;RAC3
PRKCA	Protein kinase C alpha type;PRKCA
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha isoform;PIK3CA
MRAS	Ras-related protein M-Ras;MRAS
TSC2	Tuberin;TSC2
AKT2	RAC-beta serine/threonine-protein kinase;AKT2

EGFR	Epidermal growth factor receptor;EGFR
PRKCD	Protein kinase C delta type;PRKCD
PIK3C2B	Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing beta polypeptide;PIK3C2B
Breast Cancer Regulation by Stathmin1	
Gene Symbol	Gene Name
ADCY1	Adenylate cyclase type 1;ADCY1
PPP2R5B	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit beta isoform;PPP2R5B
PPP2R1A	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform;PPP2R1A
PPP2R2C	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B gamma isoform;PPP2R2C
CALM3	Calmodulin;CALM3
CALM3	Calmodulin;CALM3
CDC42	Cell division control protein 42 homolog;CDC42
CCNE1	G1/S-specific cyclin-E1;CCNE1
CCNE2	G1/S-specific cyclin-E2;CCNE2
GNB1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1;GNB1
GNB2	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2;GNB2
GNAI2	Guanine nucleotide-binding protein G(i), alpha-2 subunit;GNAI2
ARHGEF16	Rho guanine nucleotide exchange factor 16;ARHGEF16
ARHGEF7	Rho guanine nucleotide exchange factor 7;ARHGEF7
ITPR3	Inositol 1,4,5-trisphosphate receptor type 3;ITPR3
PRKCE	Protein kinase C epsilon type;PRKCE
PRKCZ	Protein kinase C zeta type;PRKCZ

E2F1	Transcription factor E2F1;E2F1
E2F5	Transcription factor E2F5;E2F5
CDKN1A	Cyclin-dependent kinase inhibitor 1;CDKN1A
CDKN1B	Cyclin-dependent kinase inhibitor 1B;CDKN1B
PLCB1	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-1;PLCB1
PLCB3	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-3;PLCB3
PPP2R3A	Serine/threonine-protein phosphatase 2A regulatory subunit B'' subunit alpha;PPP2R3A
PRKCA,PRKACA	Protein kinase C alpha type;PRKCA
SHC1	SHC-transforming protein 1;SHC1
STMN1	Stathmin;STMN1
MAPK3	Mitogen-activated protein kinase 3;MAPK3
PPP1R14B	Protein phosphatase 1 regulatory subunit 14B;PPP1R14B
MAP2K2	Dual specificity mitogen-activated protein kinase kinase 2;MAP2K2
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha isoform;PIK3CA
MRAS	Ras-related protein M-Ras;MRAS
TUBA1A	Tubulin alpha-1A chain;TUBA1A
ARHGEF1	Rho guanine nucleotide exchange factor 1;ARHGEF1
CALM3	Calmodulin;CALM3
GNG11	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-11;GNG11
ARHGEF17	Rho guanine nucleotide exchange factor 17;ARHGEF17
PRKACA	cAMP-dependent protein kinase catalytic subunit alpha;PRKACA
CAMK2G	Calcium/calmodulin-dependent protein kinase type II gamma chain;CAMK2G

TUBB3	Tubulin beta-3 chain;TUBB3
PRKCD	Protein kinase C delta type;PRKCD
PIK3C2B	Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing beta polypeptide;PIK3C2B
Hereditary Breast Cancer Signaling Genes	
Gene Symbol	Gene Name
CCND1	G1/S-specific cyclin-D1;CCND1
FANCE	Fanconi anemia group E protein;FANCE
DDB2	DNA damage-binding protein 2;DDB2
RAC3	Nuclear receptor coactivator 3;NCOA3
HDAC3	Histone deacetylase 3;HDAC3
HDAC6	Histone deacetylase 6;HDAC6
E2F1	Transcription factor E2F1;E2F1
CDKN1A	Cyclin-dependent kinase inhibitor 1;CDKN1A
PTEN	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN;PTEN
RAC3	Ras-related C3 botulinum toxin substrate 3;RAC3
PALB2	Partner and localizer of BRCA2;PALB2
MSH6	DNA mismatch repair protein Msh6;MSH6
SLC19A1	Folate transporter 1;SLC19A1
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha isoform;PIK3CA
SMARCA2	Probable global transcription activator SNF2L2;SMARCA2
SMARCA4	Probable global transcription activator SNF2L4;SMARCA4
MRAS	Ras-related protein M-Ras;MRAS
UBC	Ubiquitin;UBC
POLR2E	DNA-directed RNA polymerases I, II, and III subunit

	RPABC1;POLR2E
POLR2K	DNA-directed RNA polymerases I, II, and III subunit RPABC4;POLR2K
AKT2	RAC-beta serine/threonine-protein kinase;AKT2
RFC3	Replication factor C subunit 3;RFC3
PIK3C2B	Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing beta polypeptide;PIK3C2B
GADD45B	Growth arrest and DNA-damage-inducible protein GADD45 beta;GADD45B
UBC	Ubiquitin;UBC
Estrogen-Dependent Breast Cancer Signaling Genes	
Gene Symbol	Gene Name
CCND1	G1/S-specific cyclin-D1;CCND1
HSD17B12	Estradiol 17-beta-dehydrogenase 12;HSD17B12
HSD17B14	17-beta-hydroxysteroid dehydrogenase 14;HSD17B14
CREB3L4	Processed cAMP-responsive element-binding protein 3-like protein 4;CREB3L4
RAC3	Nuclear receptor coactivator 3;NCOA3
SP1	DAN domain family member 5;DAND5
RAC3	Ras-related C3 botulinum toxin substrate 3;RAC3
MAPK3	Mitogen-activated protein kinase 3;MAPK3
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha isoform;PIK3CA
SP1	Transcription factor Sp1;SP1
MRAS	Ras-related protein M-Ras;MRAS
RELA	Transcription factor p65;RELA
AKT2	RAC-beta serine/threonine-protein kinase;AKT2
EGFR	Epidermal growth factor receptor;EGFR

IGF1R	Insulin-like growth factor 1 receptor beta chain;IGF1R
PIK3C2B	Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing beta polypeptide;PIK3C2B
CREB1	cAMP response element-binding protein;CREB1

Supplementary Table 7. Relationship between expression of Trim 24 and ER in surgical specimens of breast cancer

		Expression of ER			P value
		Negative	Positive	Total	
Trim 24	-/+	10 (20%)	18 (34%)	28 (27.2%)	P=0.15
	++	9 (18%)	12 (22.6%)	21 (20.4%)	
	+++	31 (62%)	23 (43.4%)	54 (52.4%)	
Total		50 (100%)	53 (100%)	103 (100%)	

- The expression patterns of these two molecules in the breast cancer samples were determined and summarized. The correlation between antibodies with Trim 24 and ER was analyzed using SPSS Pearson Chi-Square test (P=0.15). No correlation between Trim 24 and ER (P= 0.15). A p-value of less than 0.05 was set as the criterion for statistical significance.
- -/+ Indicates expression of negative and low. ++ Indicates expression of median. +++ Indicates expression of high.

Supplementary Figure 1 | TRIM24 interacts with unmethylated H3K4

through its PHD finger. a, Sequence alignment of PHD fingers of TRIM24, BHC80 and ING1: zinc coordination residues of PHD colored in yellow; critical residues for H3K4me0 recognition highlighted in green. TRIM24 PHD shares only 20% identity with ING1 PHD. **b**, Left panel, co-immunoprecipitation of ectopically expressed TRIM24 and TRIM24 Δ C (deletion of the PHD-Bromo domains) with endogenous H3 in HEK293T cells. Right panel, recombinant GST-tagged TRIM24 PHD-Bromo, purified from bacteria, interacts with native histone proteins that purified from mouse liver. **c**, Microarrays spotted with the indicated histone peptides were probed with GST-tagged TRIM24 PHD-Bromo. Red spots indicate positive binding of TRIM24 PHD-Bromo. **d**, Biotinylated peptide pulldown assays indicate the *in vitro* binding of recombinant TRIM24 PHD finger to histone peptides. **e**, GST-pulldown assays indicate the *in vitro* binding between recombinant proteins and native histone proteins. Western blot was analyzed with anti-H3K4me3 or anti-H3K9me2 antibodies first followed by anti-H3 and anti-GST antibodies. Bacterial purified GST-tagged proteins were analyzed by SDS-PAGE followed by Colloidal Blue staining.

Supplementary Figure 2 | Detailed interactions at the interface between the

extended PHD finger and the bromodomain of TRIM24. The extended PHD finger domain is colored cyan, the bromodomain is colored salmon and the linker is colored yellow. Secondary elements are labeled accordingly. Residues L913, F914 and Y916 from the α Z helix and V831, F848 and F869 from the PHD finger

form a hydrophobic intermolecular interface, while positively charged long side chain residues of K905, R906 and R910 from the α Z helix and K949 from the α A helix of bromodomain, protrude into the PHD finger domain, thereby further stabilizing the PHD-Bromo unit by a network of hydrogen bonds and salt bridge interactions.

Supplementary Figure 3 | The structure of the complex between TRIM24 PHD-Bromo and H3(1-10)K4 peptide. **a**, 2Fo-Fc omit map of the peptide segment 1-10 is contoured at 1σ level and colored green. The bound histone peptide is shown in a stick representation with residue positions labeled in blue. **b**, Electrostatic surface of TRIM24 PHD-Bromo, with bound histone H3(1-10)K4 peptide shown in stick representation.

Supplementary Figure 4 | Mutation of TRIM24 C840W interferes with the binding of TRIM24 PHD-Bromo to H3 N-terminal tail. Biotinylated peptide pulldown assays to detect the binding between H3(1-21) peptide and recombinant WT or mutant (C840W or F979A/N980A) TRIM24 PHD-Bromo.

Supplementary Figure 5 | TRIM24 bromodomain interacts with several acetylated histone peptides. **a**, NMR-based chemical shift monitoring of H3(13-32)K23ac (top panel) and H3(13-32)K27ac (bottom panel) peptide binding to TRIM24 bromodomain. Overlaid ^1H , ^{15}N -HSQC spectra of TRIM24 bromodomain

in the free-state (black) and bound to H3(13-32)K23ac (magenta) or H3(13-32)K27ac (red) at 1:3 ratio were shown in the left panels. A zoomed view of a region showing evident non-overlapped peaks between free and bound forms is shown in the right panels. The observed complexation shifts of cross peaks of TRIM24 bromodomain on proceeding from free to peptide-bound forms are indicative of direct interactions between TRIM24 bromodomain and the added peptides. **b**, Upper panel, biotinylated peptide pulldown assays indicate the *in vitro* binding of recombinant wild-type but not the bromodomain-mutant TRIM24 PHD-Bromo to acetylated H4 peptides. H4ac contains a mixture of K5/8/12/16 acetylation. Lower panel, peptide pulldown assays for TRIM24 PHD-Bromo and several biotin-labeled acetyl lysine containing H4 peptides. TRIM24 showed binding for all the acetyl lysine-containing H4 peptides and unmodified H3 peptide, but not for unmodified H4 peptide. Immobilized peptides were shown below.

Supplementary Figure 6 | Structure of complex of TRIM24 PHD-Bromo and H3(13-32)K23ac peptide. **a**, Fo-Fc omit map of the peptide segment 22-31 is contoured at 2.5 σ level and colored green. The bound histone peptide is shown in a stick representation with residue positions labeled in blue. Residue positions from TRIM24 bromodomain are labeled in black. TRIM24 PHD-Bromo is shown in a ribbon representation, with PHD domain colored cyan and bromodomain colored salmon. **b**, Electrostatic surface view of TRIM24 PHD-Bromo with bound H3(22-29)K23ac peptide shown in stick representation.

Supplementary Figure 7 | Complex structure of TRIM24 PHD-Bromo and H3(23-31)K27ac peptide. **a**, Detailed interactions between TRIM24 PHD-Bromo and H3(23-31)K27ac peptide in the crystal structure of its complex. **b**, Electrostatic surface view of TRIM24 PHD-Bromo with bound H3(23-31)K27ac peptide shown in stick representation.

Supplementary Figure 8 | Structure of complex of TRIM24 PHD-Bromo and H4(14-19)K16ac peptide. **a**, Detailed interactions between TRIM24 PHD-Bromo and H4(14-19)K16ac peptide in the crystal structure of its complex. The bromodomain is colored in salmon and the bound peptide is colored in yellow. **b**, Electrostatic surface view of TRIM24 PHD-Bromo with bound H4(14-16)K16ac peptide shown in stick representation.

Supplementary Figure 9 | Peptide orientation on the surface of the TRIM24 PHD-Bromo dual domain. **a**, Positioning of A1 to S10 of H3(1-10)K4 and T22 to A29 of H3(13-32)K27ac on the surface of the TRIM24 PHD-Bromo dual domain based on crystal structural information. Note that both peptides are oriented in opposite directions. **b**, Positioning of A1 to S10 of H3(1-10)K4 and G14 to K16ac of H4(14-19)K16ac on the surface of the TRIM24 PHD-Bromo dual domain based on crystal structural information. Note that the peptides are also oriented in opposite directions.

Supplementary Figure 10 | TRIM24 interacts with ER α in a ligand dependent manner. Top panel, endogenous co-immunoprecipitation of TRIM24 and ER α in hormone-deprived MCF7 cells, with or without estradiol (E₂)

treatment. Bottom panel, GST-pulldown assays of recombinant full-length GST-TRIM24 and recombinant ER α in the absence and presence of E₂.

Supplementary Figure 11 | TRIM24 is recruited with ER α to the ERE sites in a ligand-dependent manner. Parental MCF7 cells were hormone deprived for 96 h prior E₂ treatment. ChIP experiments were performed to determine the binding of ER α and TRIM24 at the ERE sites of the *PR* and *pS2* genes with 6 h EtOH or E₂ treatment. The antibody/protein-bound DNA was purified and quantified by qPCR to detect actual percent binding of input in the indicated regions. Each bar represents the averaged results with error bars showing standard deviations. PR-221, PR-205 and PR-95 indicate 221, 205 or 95kb upstream the transcription start site (TSS) of the *PR* gene. PR+4: 4kb downstream TSS of the *PR* gene. Note that PR-221 and PR-205 regions contain several ERE sites while PR-95 and PR+4 are ERE negative regions. pS2-ERE: proximal ERE site of the *pS2* gene, close to TSS site. cds: 3' coding sequence.

Supplementary Figure 12 | Quantified H3K4me2 and H3K4me3 levels decreased at the ERE sites after E₂ treatment. Parental MCF7 cells were hormone deprived for 96 h prior E₂ treatment. ChIP experiments were performed to determine the levels of H3K4me2 and H3K4me3 at the *GREB1* ERE (distal and proximal) and negative control (+54) sites with 6 h E₂ treatment. The antibody/protein-bound DNA was purified and quantified by qPCR to detect actual percent binding of input in the indicated regions. Each bar represents the averaged results and error bars show standard deviations.

Supplementary Figure 13 | H3K4me2 and H3K4me3 levels remain unchanged at the ERE sites in response to estrogen treatment. Parental MCF7 cells were hormone deprived for 96 h prior E₂ treatment. ChIP experiments were performed to determine the levels of H3K4me2 and H3K4me3 at the ERE sites of the *PR* gene after 6 h E₂ treatment. The antibody/protein-bound DNA was purified and quantified by qPCR to detect actual percent binding of input in the indicated regions. Levels of H3K4me2 and H3K4me3 are normalized for H3 recovery. Each bar represents the average results and error bars show standard deviations.

Supplementary Figure 14 | Global ER α and TRIM24 binding sites are altered with estrogen addition. **a**, Venn diagrams of ER α and TRIM24 (T24) sites of chromatin binding, as revealed by ChIP-sequencing analysis of MCF7 cells with (+E) or without (-E) E₂ treatment for 6 h. **b**, Genome wide binding sites of ER α , TRIM24 and FOXA1²⁸ and their overlap, plus and minus E₂. **c**, TRIM24- and ER α -bound target genes within 10 kb of their interaction sites (as in b, +estrogen): total number of genes; gene differentially regulated by E₂ (ER-regulated²⁹) in each set; percent of total regulated by E₂. Statistical significance of ER-regulated gene enrichment was determined for each set: TRIM24 only, p-value>0.9; ER only, p-value<0.5; TRIM24/ER-shared, p-value<0.001. **d**, Normalized H3K4me2 signals within a window of 800 bp, centered at genome

wide Trim24 binding sites (top) and FOXA1 binding sites²⁸ (bottom) (designated as 0), in the presence (blue line) or absence (red line) of E₂ treatment.

Supplementary Figure 15 | ChIP-seq of H3K4me2 levels and TRIM24 chromatin interactions reveals TRIM24 binding to regions depleted of H3K4me2 and no estrogen-dependent gain of H3K4me2 at these sites. a,

Estrogen-dependent binding of TRIM24 occurs at the proximal ERE of *GREB1* where H3K4me2 levels are depleted and unchanged. **b,** Estrogen-dependent binding of TRIM24 occurs at the distal ERE of *GREB1* where H3K4me2 levels exist prior to estrogen-treatment and are depleted with estrogen addition. **c,** Estrogen-dependent binding of TRIM24 occurs at the ERE of *IGFBP4* where H3K4me2 levels are specifically depleted in response to estrogen treatment.

Supplementary Figure 16 | Loss of TRIM24 impairs ER α -mediated transcription activation. a,

Hormone deprived parental, stable control shRNA depleted (shControl) and TRIM24 shRNA depleted (shTRIM24) MCF7 cells were treated with E₂ for 3h and 6h. RNA was isolated and analyzed by qRT-PCR to measure RNA levels of *TRIM24*, *PR* and *pS2*. The indicated RNA levels were normalized to *GAPDH* RNA levels and the RNA levels of untreated parental MCF7 were set as one fold. Each bar represents the average results for three independent RT-PCR experiments and error bars represent the standard deviations. **b,** Western blot analysis of TRIM24 and ER α protein levels in stable, control (shControl) and TRIM24-depleted (shTRIM24) MCF7 cells. **c,** ChIP for

histone modifications at distal ERE site of *GREB1* with 6 h E₂ treatment in stable shControl and shTRIM24 MCF7 cells. Levels of histone modifications are normalized for H3 recovery. Each bar represents averaged results; error bars show standard deviation.

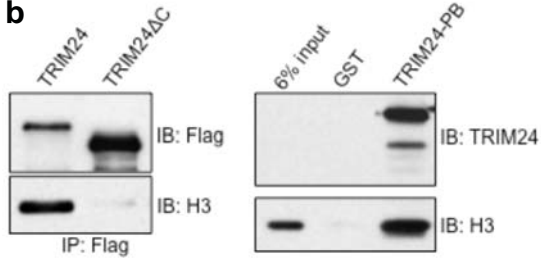
Supplementary Figure 17 | Depletion of TRIM24 leads to a significant decrease in ER α binding to ERE sites. Stable control shRNA and shTRIM24 MCF7 cells were hormone deprived for 96 h prior E₂ treatment. ChIP experiments were performed to determine the binding of ER α and TRIM24 at ERE sites with 6 h E₂ treatment. The antibody/protein-bound DNA was purified and quantified by qPCR to detect actual percent binding of input in the indicated regions of the PR (top) and pS2 (bottom) genes. Each bar represents the average results and error bars show standard deviations.

Supplementary Figure 18 | LSD1 binding to EREs is induced by estrogen treatment. Parental MCF7 cells were hormone deprived for 96 h prior E₂ treatment. ChIP experiments were performed to determine the levels of LSD1 bound at the *GREB1* ERE (distal and proximal) and negative control (+54) sites with 6 h E₂ treatment. The antibody/protein-bound DNA was purified and quantified by qPCR to detect actual percent binding of input in the indicated regions. Each bar represents the averaged results and error bars show standard deviations.

a

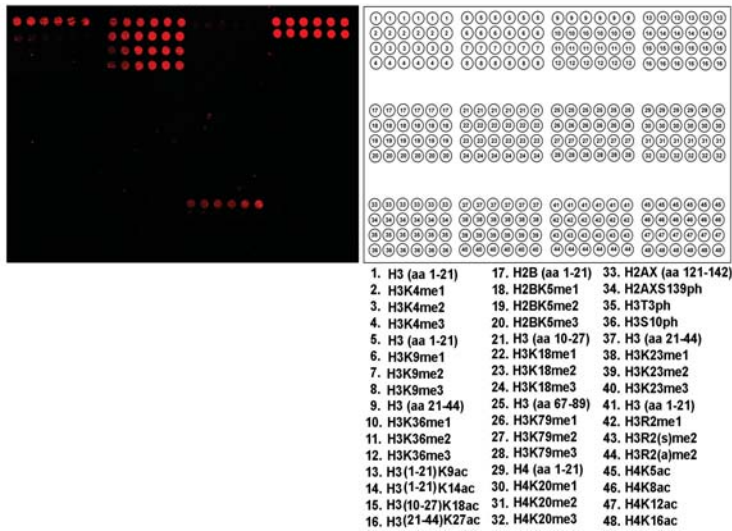
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BHC80-PHD	488-535	EDFCSVCRKS--GQLLMCDT--CS-RVYHLDCLDPPLKTIPKGMWICPRCQDQ	48
ING1-PHD	353-402	PTYC-LCNQVSYGEMIGCDNDECPIEFWHFSCVG--LNHKPKGKWCPCRCGE	50

b

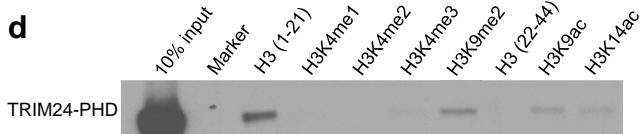


c

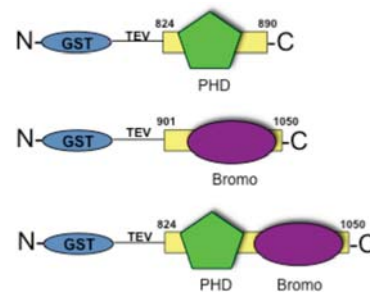
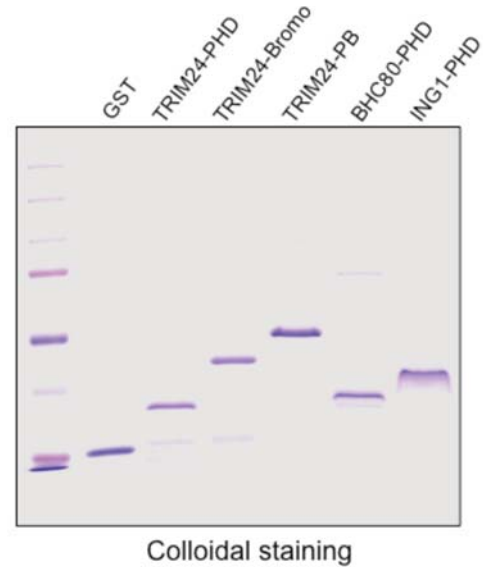
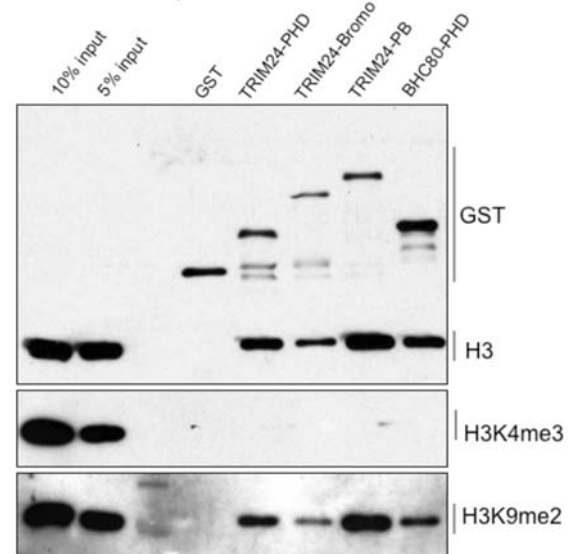
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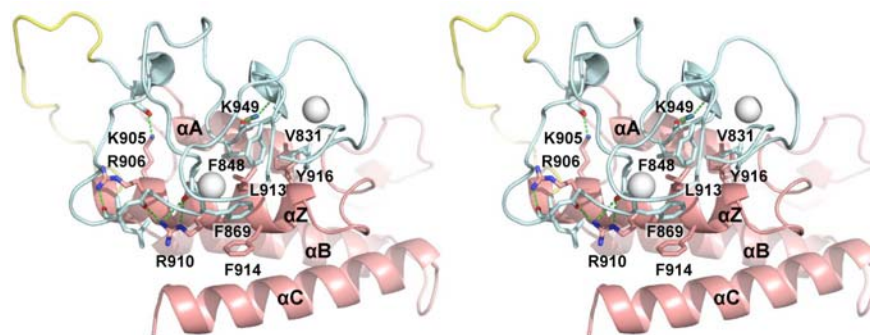


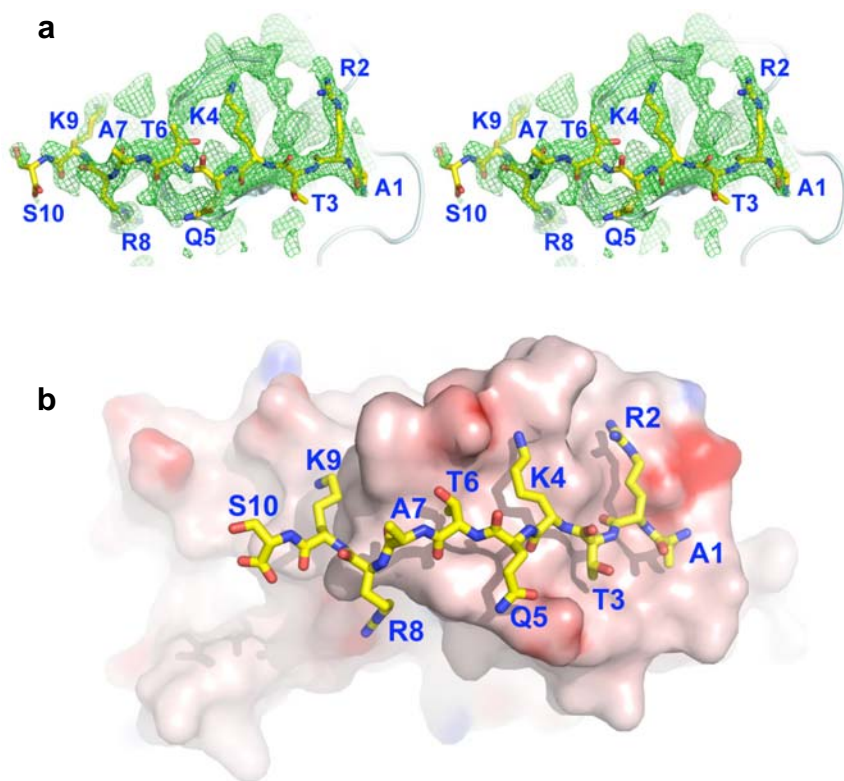
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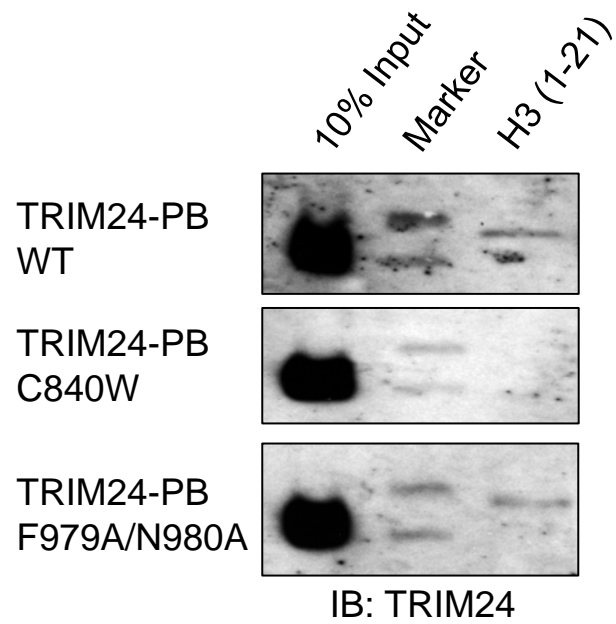


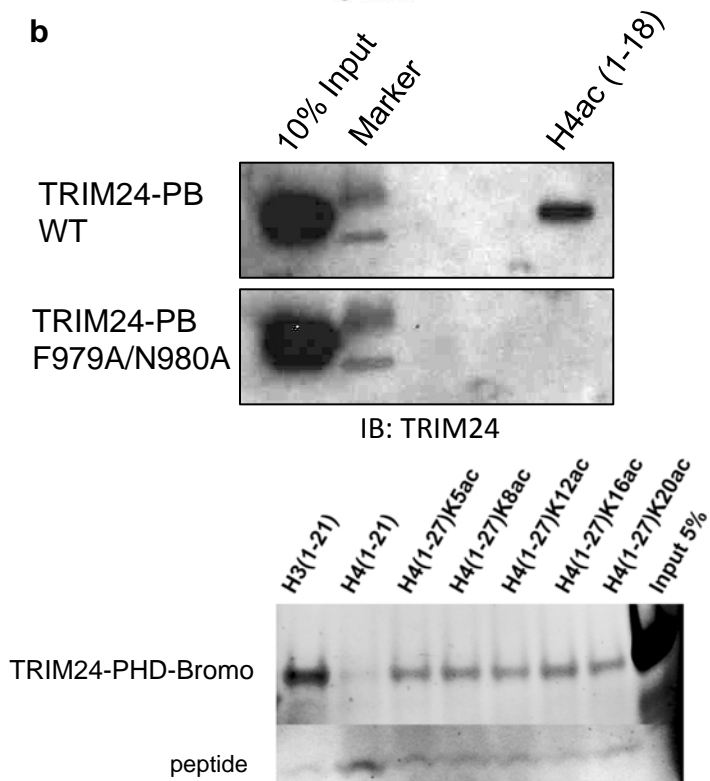
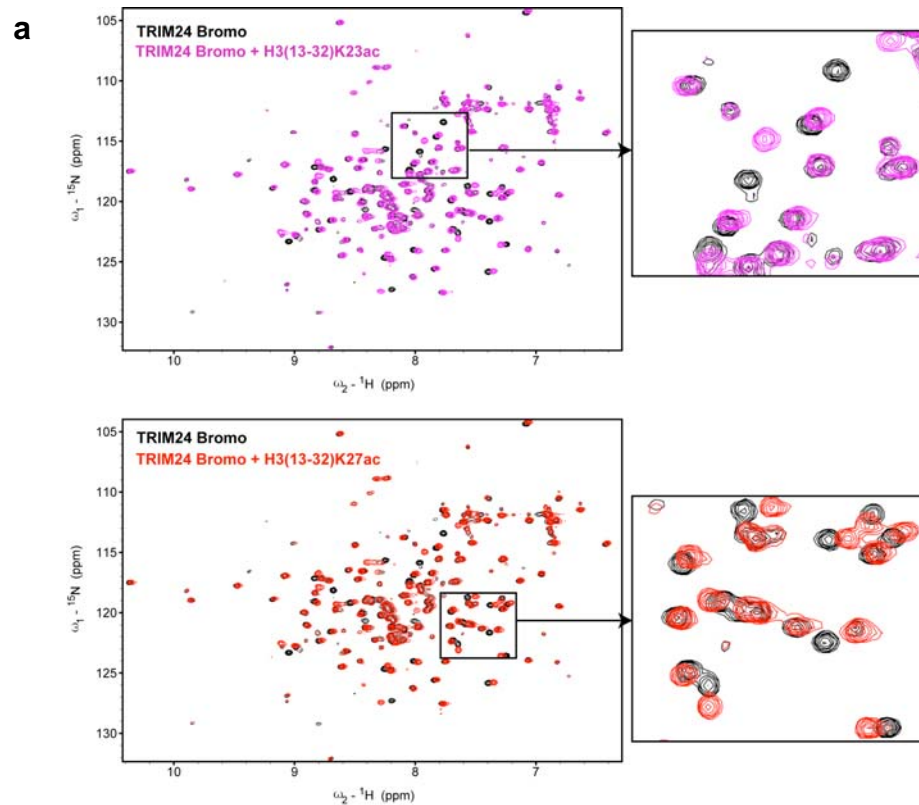
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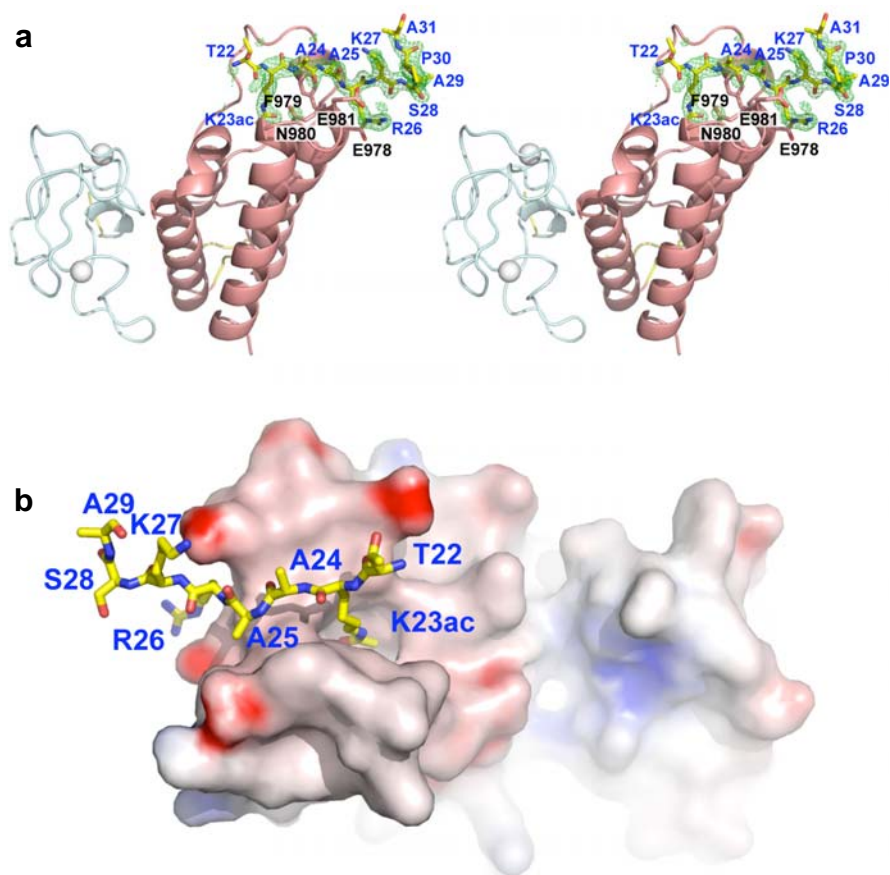


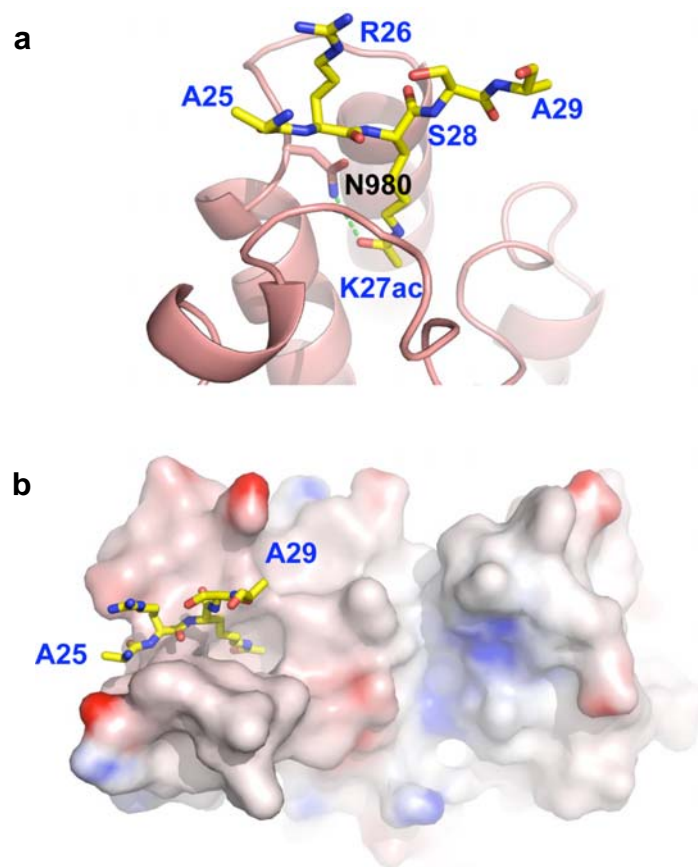


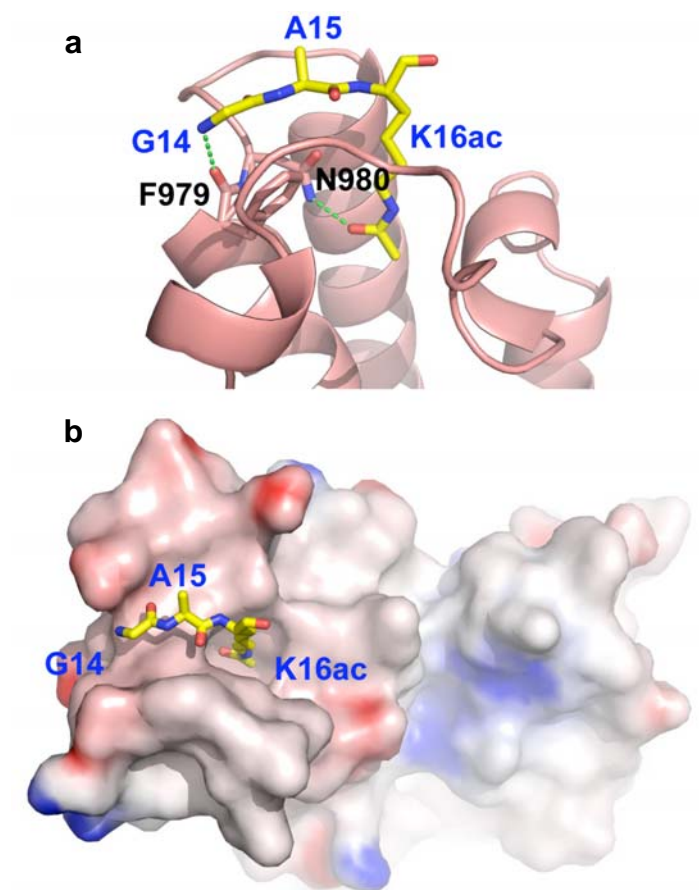


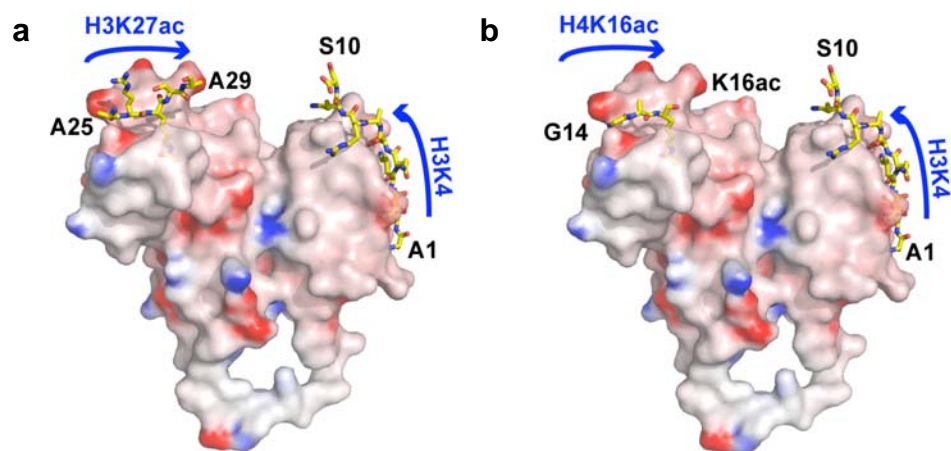


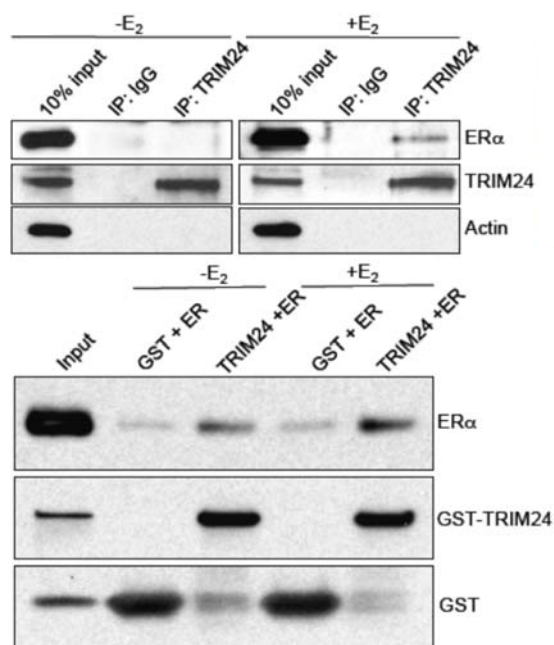


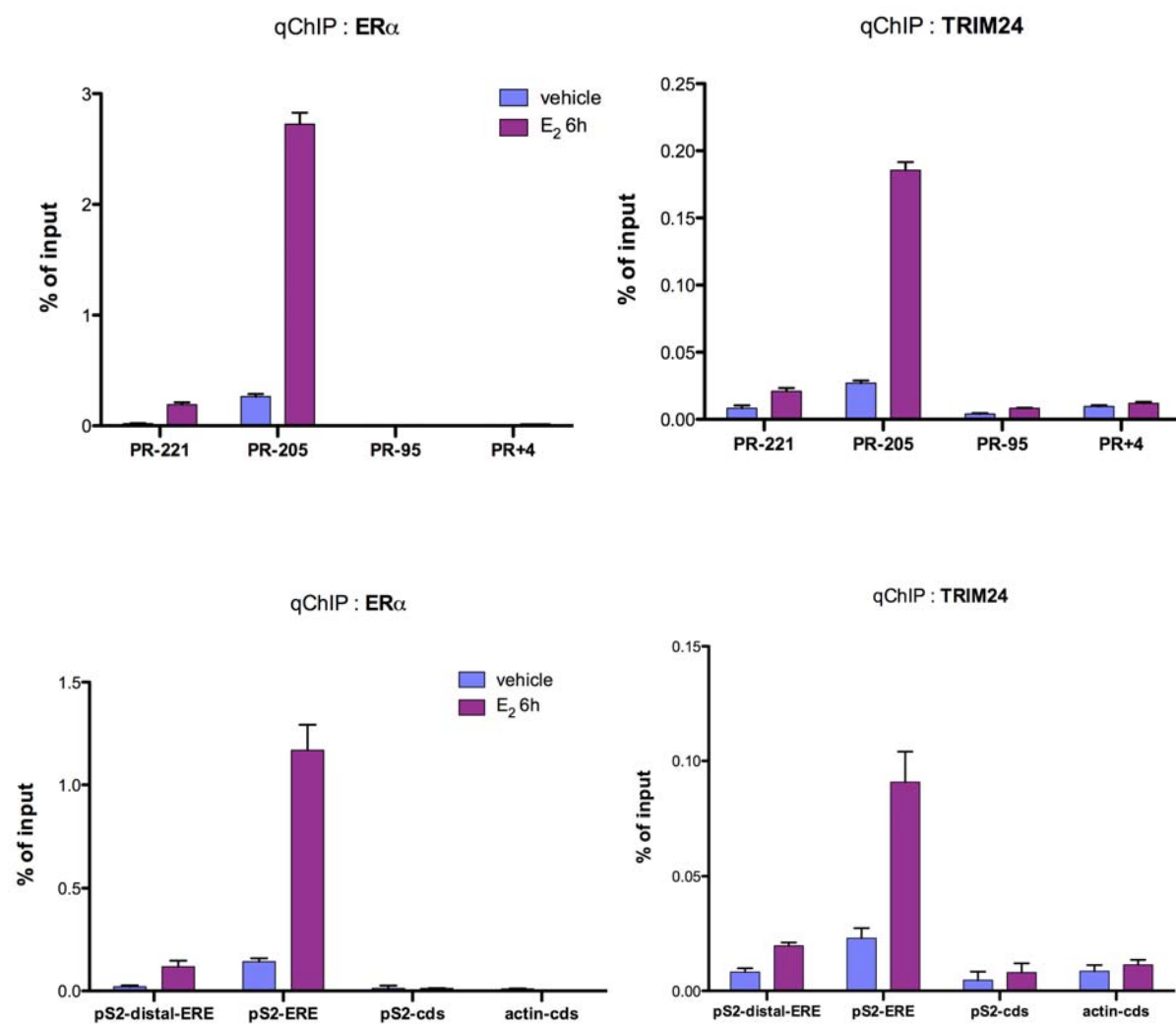


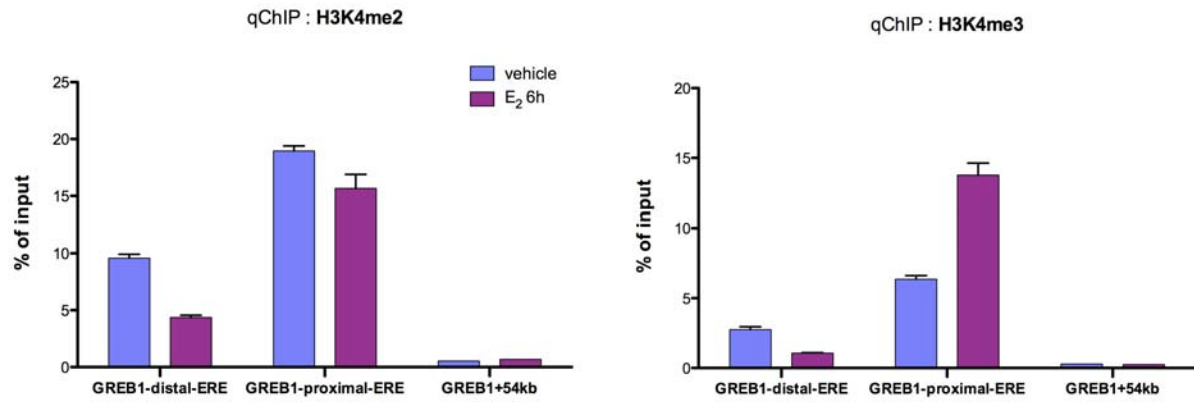


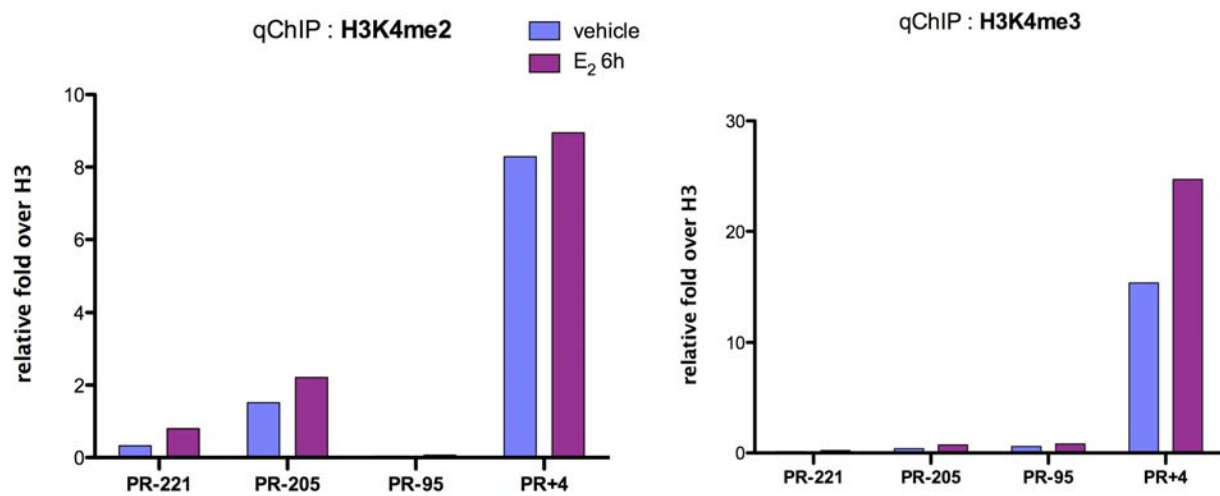


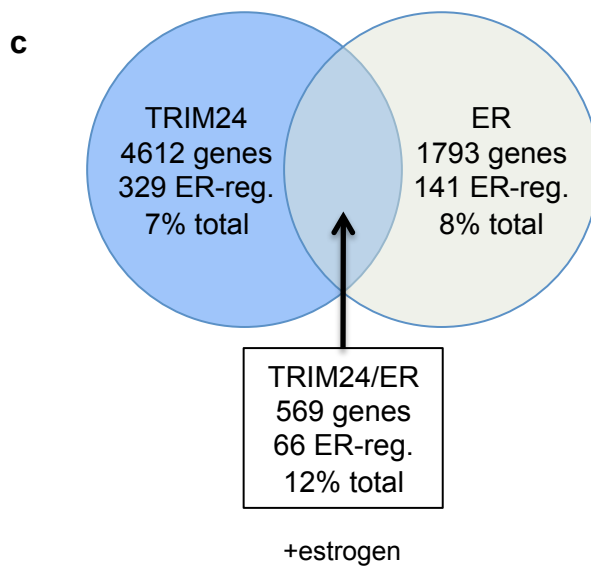
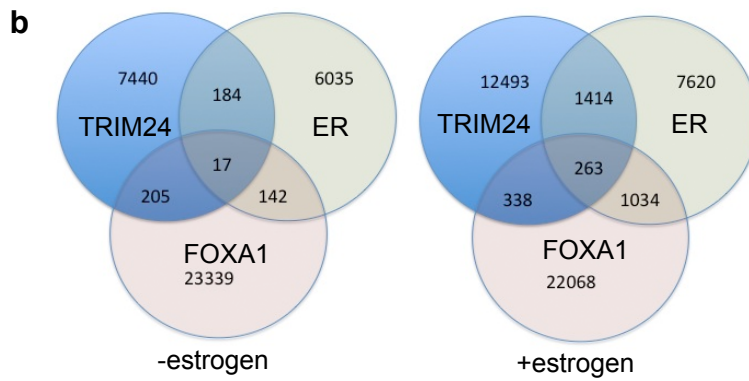
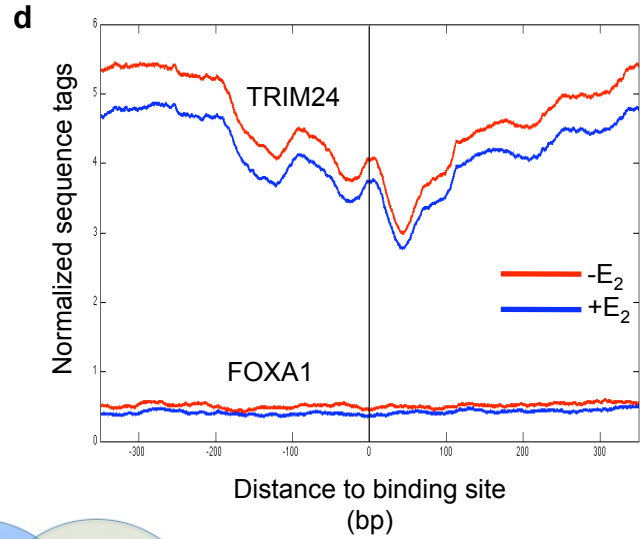
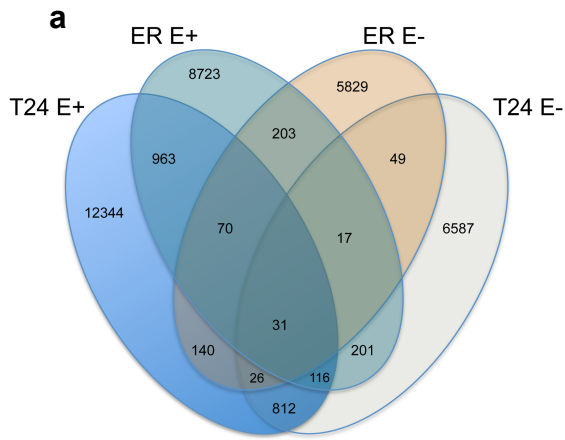




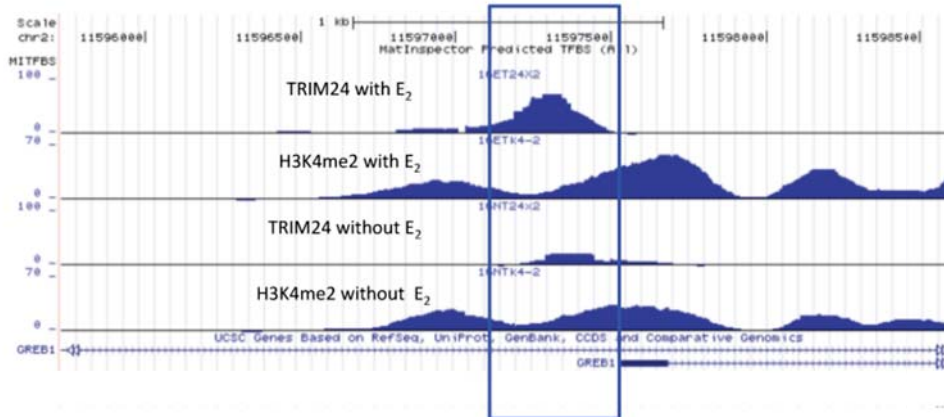




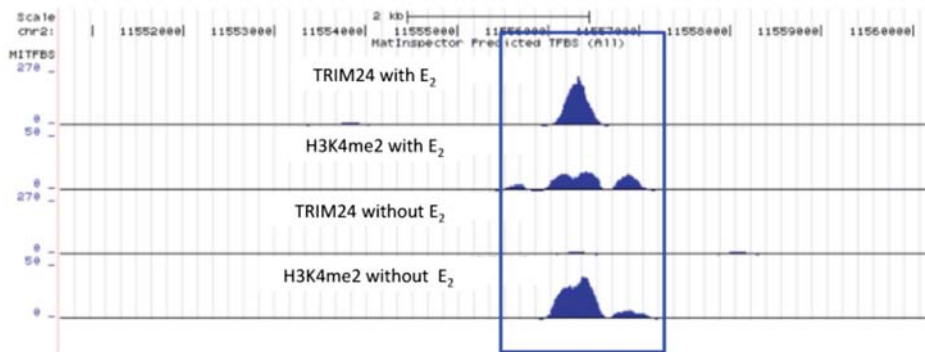




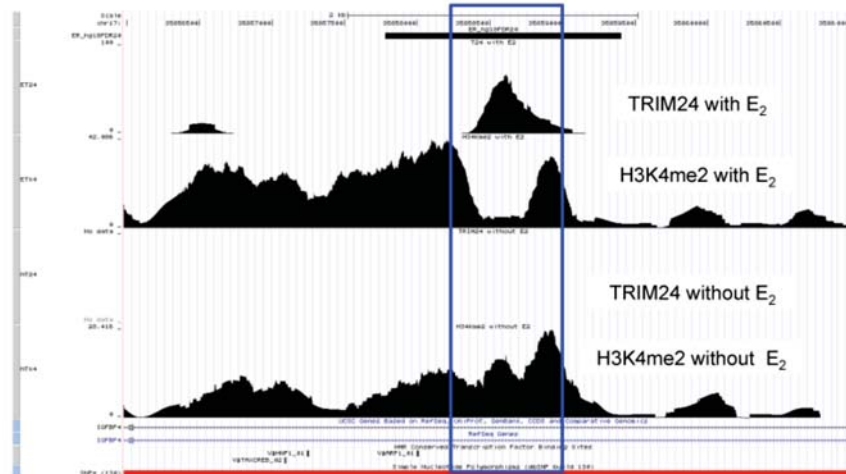
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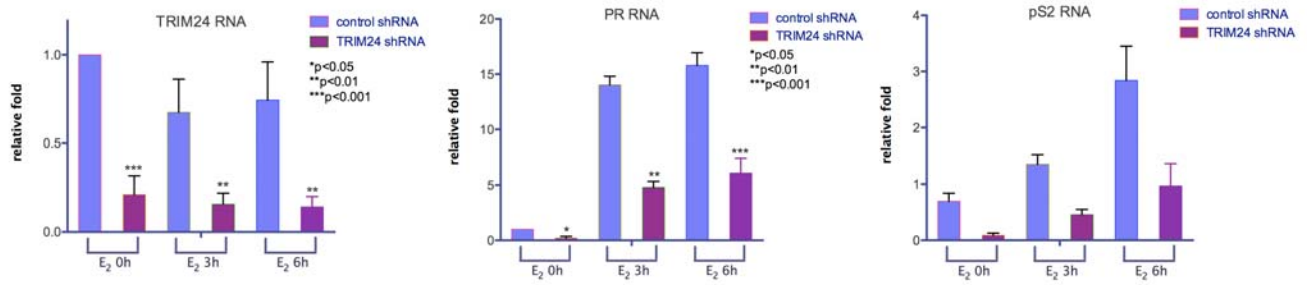
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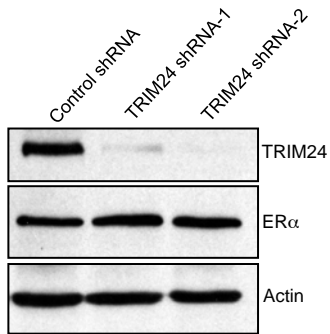
c IGFBP4 ERE



a



b



c

